Abstract

The medicinal plants played an important role in the discovery of new drugs. A lot of natural product was used as a pharmaceutical product in the treatment of many diseases. Diabetes mellitus and arthritis are the most common health problem in the World, and the prevalence of this disease is rapidly increasing. Despite the availability of synthetic drugs, herbal formulation is desirable, and hence they are investigated with renewed interest all over the World. Therapy with herbal drugs is an older traditional system and plants have been used over the years for the treatment of arthritis and diabetes mellitus. A lot of researchers and scientists to evaluate the biological activity, less toxicity and safety of medicinal plants to promote their use for the development of new pharmaceuticals which may be more potent than currently used for the diabetes and arthritis.

Among the thousands of Indian medicinal plants *Aegle marmelos* Correa, *Moringa oleifera* Lam, *Paederia foetida* Linn and *Melastoma malabathricum* Linn were selected for the present study on the basis of their use in Ayurvedic & traditional medicine. The literature survey revealed that these plants were used in different polyherbal preparations and extract used to treat the hypoglycemia and arthritic disorder lack scientific experimental evidence of their antidiabetic and antiarthritic activity. In view of the above facts, it was thought worthwhile to take the phytopharmacological screening of these plants for their antidiabetic and antiarthritic activity using different in-vivo models. Concerning to the above situation, the present investigation was taken to generate medicinal use of the medicinal plants. The present study was divided into five sections, as follows viz. Part A-D.

The stem of *Aegle marmelos* (Corr.) (Part A) was collected from the Herbal garden, Department of Pharmaceutical Sciences, SHIATS, Allahabad, India and the plant material was authenticated by the Dr. Imran Kazmi, Siddhartha Institute of Pharmacy, Dehradun, Uttarakhand, India and standard herbarium specimens deposited in the same department for further use. The plant materials were subjected to the shade dried and crushed with the help of the grinder to make the powered.

A thin section of the *Aegle marmelos* (Corr.) stem showed small, thick-walled sclerenchyma single layer of living cells, thickened and covered with waterproof a layer called as the cuticle. The layers of the epidermis are very closely packed. The t.s. of the stem contain the xylem, phloem and cambium. The xylem found in the center part of the vascular bundle and phloem found in the outside of the vascular bundle. The cambium separates the xylem and phloem. Xylem of vessels with simple end-walls, partially developed. The smaller vessels constituting the protoxylem and the bigger ones constituting the metaxylem lie away from the center. The protoxylem consists of annular, spiral and scalariform vessels, and the metaxylem of reticulate and pitted vessels. Cambium consists of 2-3 layers of thin walled and rectangular, and is arranged in radial rows. Fewer layers of fairly massive polygonal or readily elongated parenchymatous cell packed with yellow-brown masses (pigments).

The successive extract was subjected to the chemical test for identification of the chemical present in the particular solvent. Preliminary phytochemical screening of the various extracts revealed the presence of the alkaloids, carbohydrates, glycosides, phenolic compound, tannin, saponin, sterol, gum and mucilage were present in the *Aegle marmelos* (Corr.).
The plant material of the *Aegle marmelos* (Corr.) (5 kg) dried, powdered and subjected to extraction in the soxhlet apparatus using the methanolic extract. The methanolic extract was subjected to the column chromatography; the methanolic extract was separated two compounds and these separated compounds was characterized as the umbelliferone β-D-galactopyranoside and umbelliferone diglucoside by the help of the $^1$H and $^{13}$C NMR, Mass, FTIR. Umbelliferone β-D-galactopyranoside (UFG) and umbelliferon-α-D-glucopyranosyl-(2I→1II)-α-D-glucopyranoside (UFD) were evaluated for antidiabetic (oral glucose tolerance test and streptozotocin induced model), antiarthritic (turpentine oil induced model, formaldehyde induced model and complete freund’s arthritis induced model) activity at different doses.

UFG orally treated rats (Part A-1) exhibited normal behavior till doses 100 mg/kg, these groups behave normally on touching and pain response. There was no lethality or any toxic reactions found with the selected dose until the end of the study. The acute and chronic anti-inflammatory activity of the UFG was evaluated against the turpentine oil induced joint edema, formaldehyde and CFA induced arthritic methods. Different doses of the UFG administered orally, significantly inhibited the joint edema developed by the turpentine oil at dose dependent manner. The significant inhibitory effect of UFG was observed in the formaldehyde induced proliferative global edematous arthritis when administered orally. CFA induced arthritic rats treated with different doses of the UFG significantly decreased the humoral immune response in the both types of the reactions. CFA induced arthritic rats showed decline in the body weight throughout the study. UFG significantly decrease the body weight due to increase the absorption of the glucose and leucine through the intestine and reduced the distress caused by the bacterial induced arthritis. During the arthritic condition, CFA activates the inflammatory mediators, ROS in large amount and start the generation of the free radical in the inflamed area to damage of the tissue. ROS and free radical altered the activity of the endogenous antioxidant defenses, start the production of the superoxide, H$_2$O$_2$ and impairment the destruction of the affected joint such as synovial fluid, cartilage and other articular constituents. CFA induced rats treated with different doses of the UFG significantly increased the level of the antioxidant enzyme such as SOD, GSH, GPx and deceased the level of the MDA. CFA induced arthritic rats treated with the UFG significantly decreases the leukocyte count by stimulation of the immune system against the invading antigens and showing the immune modulating effect. ESR is the number of the cells and concentration of proteins, especially fibrinogen, alpha and beta globins. ESR was an estimate of the suspension stability of RBC’s in plasma. The ESR counted significantly decreased by the treatment of the UFG justified the antiarthritic effect of the drug. The level of the WBC increased mild to moderate is the common feature of the inflammatory reaction. WBC level was increased in the arthritic condition due to microbial infection and releasing the IL-2B inflammatory cells. IL-2B increased the production of the granulocyte and macrophage colony stimulating factors. UFG significantly decreased the level of the WBC by decreasing the level of the inflammatory mediator and macrophages colony stimulator factors.

Different doses of the UFG significantly (87±1.789) reduced the blood sugar level in oral glucose tolerance test mode when compared to the glibenclamide (88.6±1.568) showed the better utilization of the glucose. STZ induced diabetic rats treated with the different doses of the UFG significantly (123.4±2.379) reduced the blood sugar level when compared to the glibenclamide (136.4±1.99). Another biochemical parameter such as hexokinase, glucose-6-phosphate and fructose-1-6-biphosphatase significantly improved
by the treatment of the different doses of the UFG and well supported by the antidiabetic effect of the UFG. The different doses of the UFG significantly (P<0.001) decreasing the level of the total cholesterol, triglyceride, LDL cholesterol, VLDL cholesterol and increases the level of HDL cholesterol as compared to the glibenclamide. STZ induced diabetes, destroy the pancreatic insulin secreting β-cells and enhancing the level of reactive oxygen species. Three different doses of the UFG significantly decrease the level of the antioxidant enzyme SOD, CAT, GPx and increasing the level of the MDA when compared to the glibenclamide. The result suggests that the UFG has the potent antioxidant activity to reduce diabetes complication arising by the free radical generations.

Acute toxicity studies of the bioactive compound of UFD (Part A-2) revealed the non-toxic nature in the lower dose. There was no lethality or any toxic reactions found with the selected doses up to 100 mg/kg until the end of the specific study. The acute and chronic anti-inflammatory activity of the UFD was evaluated against the turpentine oil induced joint edema, formaldehyde and CFA in induced arthritic methods. Three different doses of the UFD administered orally, significantly inhibited the joint edema developed by the turpentine oil at dose dependent manner. The significant inhibitory effect of UFD was observed in the formaldehyde induced proliferative global edematous arthritis when administered orally. The result suggests that UFD significantly decrease the CFA induced humoral immune response and secondary delayed reaction was occurring due to the delayed hypersensitivity reaction. CFA induced arthritic control group rats, continuous showed the decreasing level of the body weight of the rats due to the deficient absorption of nutrients through the intestine. CFA induced arthritic rats treated with the UFD significantly improving the body weight as compared to the CFA induced arthritic control group rats. However, after this CFA induced arthritic development phase, the arthritic indexes started to decrease and finally reached less than 60% in all groups as compared to the CFA induced arthritic control group rats. CFA induced arthritic rat treated with the UFD significantly improved the level of the RBC, Hb and Decline the level of the ESR, WBC further supports its anti-arthritic effect. The different doses of the UFD increase the activity of the endogenous antioxidant such as SOD, GPx, GSH and decrease the level of the MDA.

Oral glucose tolerance test studies showed the UFD significantly (62.6±1.327) decline the level of the blood sugar when compared to the glibenclamide (69.6±1.248). STZ induced diabetes model the UFD significantly (107.4±2.731) declines the blood sugar level as compared to the glibenclamide (117.2±2.764). UFD treated group rat showed the improve level of the insulin as compared to the glibenclamide. UFD significantly increases the level of the hexokinase and decline the level of the glucose-6-Phosphate, Fructose-1-6-biphosphatase. Hyperlipidemia and hypercholesteremia are the major risk factors in the diabetes mellitus. Diabetes directly affected to the pancreatic β-cells, which inhibit the insulin secretion and production, due to dearrangements of the metabolic and regulatory process of insulin, lipids (total cholesterol and triglyceride) start to accumulate. UFD containing the potent antioxidant activity to reduce the free radical generation because the free radical plays an important role in the formation of the diabetes. UFD significantly increased the level of the SOD, CAT, GPx and increasing the level of the MDA when compared to the glibenclamide. UFD has the potent antioxidant activity and decline the diabetes complication arise from the generation of free radicals.
The stem of *Moringa oleifera* Lam (Part B) was collected from the Herbal garden, Department of Pharmaceutical Sciences, SHIATS, Allahabad, India and the plant material was authenticated by the Mr. Imran kazmi, Siddhartha Institute of Pharmacy, Dehradun, Uttarakhand, India and standard herbarium specimens deposited in the department for further use.

The plant material of the *Moringa oleifera* (Lam.) (2 kg) dried, powdered and subjected to extraction in the soxhlet apparatus using the methanolic extract. The methanolic extract was subjected to the HPTLC to identify the bioactive compound quercetin.

The acute and chronic anti-inflammatory activity of the *MO* was evaluated against the turpentine oil induced joint edema, formaldehyde and CFA induced arthritic methods. Three different doses of the *MO* administered orally, significantly inhibited the joint edema developed by the turpentine oil at dose dependent manner. The significant inhibitory effect of *MO* was observed in the formaldehyde induced proliferative global edematous arthritis when administered orally. CFA induced arthritic rat treated with the *MO* significantly inhibit the paw swelling and redness in the infected area showed the antiarthritic effect. The body weight loss was observed continuously in the CFA induced arthritic control group rats due to the deficient absorption of nutrients through the intestine. CFA induced arthritic rats treated with the *MO* significantly improving the body weight as compared to the CFA induced arthritic control group rats. CFA induced arthritic development phase, the arthritic indexes started to decrease and finally reached less than 60% in all groups as compared to the CFA induced arthritic control group rats. Our result suggests that *MO* showed significant recovery from the induced anemia with an increase in the level of RBC, Hb and decreases the level of WBC, ESR. CFA induced arthritic rat treated with the *MO* showed the significant improvement of the endogenous antioxidant till completion of the study.

Oral glucose tolerance test was performed for the identification of the glucose tolerance. Three different doses of the *MO* showed the significantly (92±1.304) decline the blood glucose level when compared to the glibenclamide (83±1.924). STZ induced diabetic rat treated with the *MO* significantly (98.6±1.435) inhibit the blood glucose level as compared to the glibenclamide (83±1.924) and the increasing the level of pancreatic insulin confirm the antidiabetic effect of the *MO* extract. Another diabetic parameter such as hexokinase, glucose-6-Phosphate and Fructose-1-6-biphosphatase showed the mark changes in the STZ induced diabetes. Another complication in the diabetes is the lipid abnormality, which was affected by the inhibition of the insulin and start the lipid accumulate on the different tissue. The *MO* extracts significantly SOD, CAT, GPx and increasing the level of the MDA when compared to the glibenclamide.

The leaves of *Paederia foetida* (Linn.) (Part C) were collected from the Herbal garden, Department of Life Sciences, Dibrugarh University, Dibrugarh, Assam, India and the plant material was authenticated by the Botanical Survey of India, Shillong, India and standard herbarium specimens deposited in the Botanical Survey of India for further use.

The plant material of the *Paederia foetida* (Linn.) (2 kg) dried, powdered and subjected to extraction in the soxhlet apparatus using the methanolic extract. The methanolic extract was subjected to the HPTLC to identify the bioactive compound ascorbic acid.

The acute and chronic anti-inflammatory activity of the *PF* was evaluated against the turpentine oil induced joint edema, formaldehyde and CFA in induced arthritic methods. Three different doses of the
*PF* administered orally, significantly inhibited the joint edema developed by the turpentine oil at dose dependent manner. The significant inhibitory effect of *PF* was observed in the formaldehyde induced proliferative global edematous arthritis when administered orally. CFA induced arthritic rat treated with the different doses of the *PF* was showing the inhibitory effect on the increased paw volume and redness in the rat hind paw and forelimbs. CFA induced arthritic rat treated with the *PF* showed the improvement in the body weight by enhanced the intestinal absorption of the nutrients and decline in the distress caused by the severity of the arthritis. However, after this CFA induced arthritic development phase, the arthritic indexes started to decrease and finally reached less than 50% in all groups as compared to the CFA induced arthritic control group rats. CFA induced arthritic rat treated with the *PF* significantly improved the level of the RBC and Hb and decline the level of the ESR and WBC. It is well known that free radicals/reactive oxygen species play an important role in inflammation. CFA induced arthritic rat treated with the *PF* significantly improved the level of the antioxidant enzyme SOD, GSH, GPx and decline the level of the MDA as compared to the arthritic control groups.

Oral glucose tolerance test was performed for the identification of the glucose tolerance of the rats. The *PF* showed the significantly (64±0.324) decline the blood glucose level when compared to the glibenclamide (68.4±1.208). STZ induced diabetic rat treated with the *MO* significantly (107.6±1.749) inhibit the blood glucose level as compared to the glibenclamide (122±1.844). STZ induced diabetic rat treated with *PF* is increasing the level of pancreatic insulin confirm the antidiabetic effect of the *PF* extract. Another diabetic parameter such as hexokinase, glucose-6-Phosphate and Fructose-1-6-biphosphatase showed the mark changes in the STZ induced diabetes. STZ induced diabetic rat treated with the *PF* extract for 28 days to produce improved the level of TC, TG, LDL, VLDL and decline of HDL cholesterol. STZ induced diabetic rat treated with *PF* extract decreased the level of MDA and improved the level of SOD, CAT and GPx and posses the antioxidant effect on the diabetes condition.

The leaves of *Melastoma malabathricum* Linn (Part D) were collected from the Herbal garden, Department of Life Sciences, Dibrugarh University, Dibrugarh, Assam, India and the plant material was authenticated by the Botanical Survey of India, Shillong, India and standard herbarium specimens deposited in the Botanical Survey of India for further use.

The plant material of the *Melastoma malabathricum* (Linn.) (2 kg) dried, powdered and subjected to extraction in the soxhlet apparatus using the methanolic extract. The methanolic extract was subjected to the HPTLC to identify the bioactive compound quercetin.

Acute toxicity of the methanolic extract of *MM* revealed the non-toxic nature of the different doses. There were no lethality or toxic reactions found in the selected group which received the different doses of the extract until the end of the experimental period. The acute and chronic anti-inflammatory activity of the *MM* was evaluated against the turpentine oil induced joint edema, formaldehyde and CFA in induced arthritic methods. Three different doses of the *MM* administered orally, significantly inhibited the joint edema developed by the turpentine oil at dose dependent manner. The significant inhibitory effect of *MM* was observed in the formaldehyde induced proliferative global edematous arthritis when administered orally. CFA induced arthritic rat treated with the *MM* showed the inhibition of chronic swelling, inflammatory autocoid and proinflammatory, which could have led to an alteration in the immunological situation during the delayed phase of the response. CFA induced arthritic rat treated with the *MM* showed